CaliciNet Outbreak Season (2011-2012)

Norovirus Identification from one of the Nation's Smallest States

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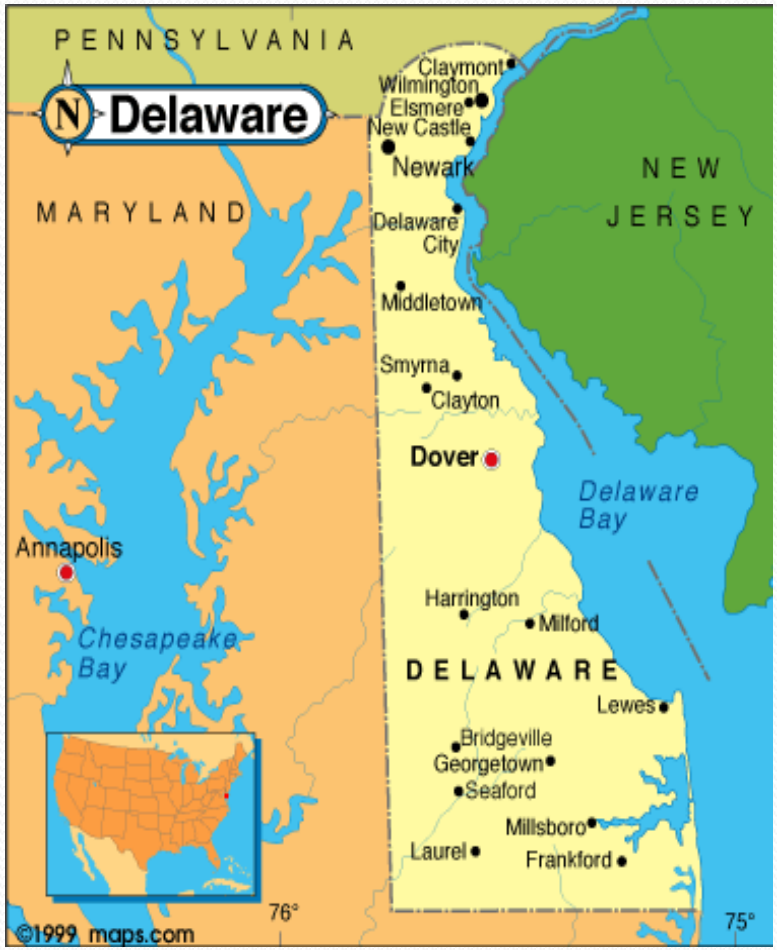
Introduction

- DPHL located in Smyrna (heart of Delaware)
- While DE ranked as the **45th** state in country for population[1] and **49th** for total area[1], DE is ranked as the **6th** state in country for population density![1]
- Hospitals and long-term care facilities commonly report outbreaks of norovirus gastroenteritis, which make up over 50% of reported outbreaks.[3]
- Norovirus introduced through ill patients, visitors, or staff, via exposure to direct/indirect fecal contamination on fomites, by eating foods prepared by ill food-handlers, by contact with body fluids or skin surfaces, or by exposure to aerosols of norovirus from vomiting persons.[3]
- **Size of state and unique courier service allow rapid submission of potential Norovirus specimens to the DPHL (~ 24 hour identification)**



Past Outbreak Season

- Began end of November 2011
- Lasted through March 2012
- Total of **16** identified outbreaks
  - One outbreak sporadic case, not epi. significant for CaliciNet
- Total **72** specimens received by lab (4.24 specimens /outbreak)
- Forty six confirmed positive samples of norovirus RNA (63.9%)

Sixteen Positive Outbreaks

- **Eight** identified as GII.4 New Orleans Strains
- **Six** identified as GII.1 Ascension208
- **One** identified as GII.6b
- **Six** outbreaks had at least **2** samples sequenced
- **Five** outbreaks had only **1** sample sequenced
- **One** outbreak was not sequenced due to low viral titer.
- **Two** outbreaks were sequenced through Region C opposed to Region D.
- *No GI norovirus detected in this outbreak season*

Norovirus Extraction and Amplification

- Samples diluted 1:10 per CDC guidelines
- Extracted using Qiagen® Viral RNA mini kit
- Protocol validated for automated extraction using Qiagen® QIAcube for outbreak season 2012-2013
- Amplification on ABI 7500FastDx system
  - Qiagen® QuantiTect® Multiplex RT-PCR Kit
  - internal control (MS2 phage)
  - used for data analysis

Norovirus Extraction and Amplification Adjustments

- Cycling conditions modified from CDC procedures to enhance amplification in multiplexed reaction.
  - 50°C for 30 mins., 95°C for 10 mins., 45 cycles of:
  - 95°C for 15 secs., 55°C for 30 secs., 72°C for 30 secs.*
  - *data analysis portion
- Gene group IV primers and controls kept in inventory incase of potential outbreak
- Adjustment of dye chemistry for qPCR multiplexing future outlook

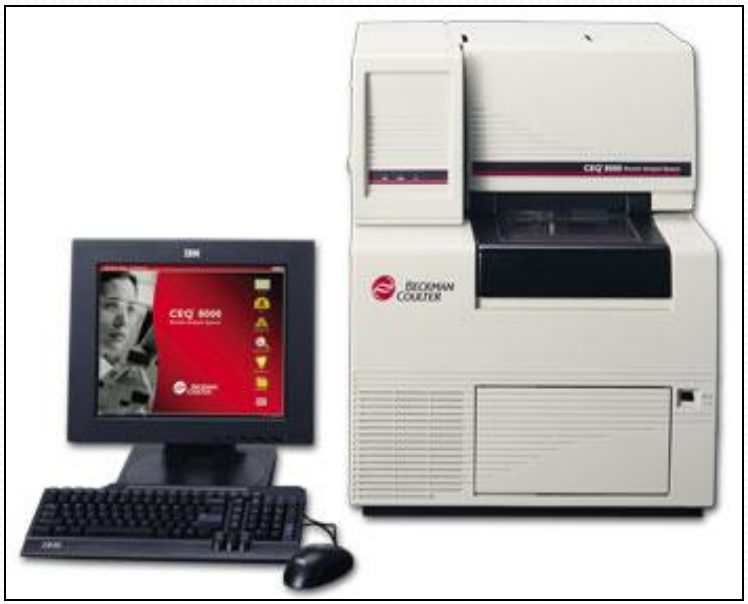
Downstream Applications

- Thermal cyclers (BioRad T100™, Eppendorf Mastercycler®, AB GeneAmp® 9700)
- Gels prepared using CDC guidelines(2%, 110 current, 60 min. run time)
- Gels stained with non-carcinogenic Biotium GelRed
- Excitation of gels for visual confirmation
- Excision of cDNA regions from gel for PCR purification
  - GE Healthcare Illustra™ GFX™ Gel Extraction and PCR Purification kit
  - Qiagen QIAquick® Gel Extraction Kit

Downstream Applications

- Quantification
  - Invitrogen™ Qubit® System using dsDNA BR assay kit
- *All kits used according to vendor guidelines*
- All sequencing performed using Beckman Coulter® guidelines and supplies
  - Cycle Sequence, GenomeLab™ DTCS quick start kit
  - Dye Terminator clean-up, Edge Bio Performa® DTR cartridges
  - Samples concentrated using LabConco® CentriVap® DNA concentrator

Sequencer and Analysis



- Beckman Coulter® CEQ™ 8000 genetic analyzer
  - 8 capillary system
- BioNumerics® version 6.6
- All analysis of samples and interpretation of data run following CDC guidelines for CaliciNet testing.

Limitations

- Issues with identifying Region D specimens (repeats)
  - Streamline process for Region D -> Region C identification
- Quantification
- Weak positives and quantification procedure provide difficulty in achieving optimal sequence results (repeats).
- Communication with facilities and within Division of Public Health
  - Delay in receiving specimens (often due to improper collection), leads to loss in viral load
  - Outbreaks not received at state lab, forwarded to reference lab (often for EIA testing).

DE Future Plans and Achievements

- Expedited resulting and achieving higher throughput for increased outbreaks by:
- New Instrumentation
  - Upgrading thermal cyclers
  - Advancing electrophoresis equipment (e-gels, docking systems, etc)
  - Upgrading quantification instruments (Nanodrop®)
- Increase number of laboratory scientists certified (+2)
- Availability of future assay technology (pyrosequencer technology)

Objectives

- Implement and validate new instruments and equipment for testing
- Train and certify new staff
- Coordinate with state and local partners to convey the importance of submitting specimens to the state lab for identification and surveillance.
- Achieve at least two acceptable sequences per outbreak that can be uploaded to the CaliciNet database.

Acknowledgments

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- Regional CaliciNet certified State Laboratories
- CaliciNet Team
  - Jan Vinje, Pd.D.
  - Leslie Barclay
  - Nicole Gregoricus

References

- 1. State of Delaware http://www.de.gov/
- 2. United States Census Bureau http://www.census.gov/
- 3. CDC: Guideline For The Prevention And Control Of Norovirus Gastroenteritis Outbreaks In Healthcare Settings, 2011.

Thank You!

Questions?